

# The Effect of Sodium Perchlorate and Ionizing Irradiation on the Thyroid Parenchymal and Pituitary Thyrotropic Cells

Zdenka Pajer, Miroslav Kališnik

Institute of Histology and Embryology, Medical Faculty, Ljubljana, Yugoslavia

46 wk AEL (CEL) at

1.2% water. Investigations  
refer to a 1-month study  
(p. 39) UF<sub>3</sub>?

**Key Words.** Thyroid parenchymal cells · Pituitary thyrotropic cells · Sodium perchlorate · Irradiation · Stereology

**Abstract.** In a 46-week-experiment on 72 female mice the influence of peroral 1.2% sodium perchlorate application, total ionizing irradiation with 8 Gy on 5 consecutive days and their interaction on the pituitary-thyroid axis was studied by histological and stereological methods. It was observed that perchlorate alone caused long-term and strong hypothyroidism with hypertrophic and hyperplastic thyroid epithelial cells as well as pituitary thyrotropic cells. When only irradiation was used, no uniform changes in the structure and function of these cells could be detected. The interaction of perchlorate and irradiation showed similar effects as estrogens alone, with some exceptions; paradoxically, minor hyperplasia of thyrotropic as well as of parafollicular cells was observed. A high percentage of the follicular cell carcinoma was found after perchlorate application and after its combination with irradiation. No medullary carcinoma was found.

## Introduction

The perchlorate ions ( $\text{ClO}_4^-$ ) inhibit the uptake of radioactive iodine in the thyroid gland [1]. Its deficiency leads to the reduced synthesis and secretion of the thyroid hormones thyroxine and triiodothyronine and to the increased concentration of pituitary thyrotropin (TSH) in the blood [2, 3]. The changed hormone levels are accompanied by hypertrophy and hyperplasia of thyroid follicular cells as well as by incipient strong hypertrophy and hyperplasia of parafollicular cell [4].

The aim of this study was to evaluate the influence of peroral application of sodium perchlorate, ionizing irradiation in low doses, and their interaction on mouse thyroid epithelial cells, on thyroid parafollicular cells, and on pituitary thyrotropic cells in an experiment of 46 weeks duration.

## Materials and Methods

Seventy-two female mice of BALB/c strain, 6 weeks of age, were randomly divided into six groups of 12 animals each. Three groups drank 1.2% sodium perchlorate solution ( $\text{NaClO}_4$  Brenat, Troponwerke, Köln), while three control groups drank tap water. Eight or ten weeks after the beginning of the experiment one perchlorate and

one control group of animals were totally irradiated with 0.8 Gy on 5 consecutive days, with the dose rate 1.45 Gy/min of gamma rays, so that each animal received a total dose of 4 Gy.

Forty-six weeks after the beginning of the experiment 42 animals were sacrificed, while 30 had died during the experiment. From the sacrificed animals the thyroid glands and pituitaries were removed for histological studies followed by stereological analysis in order to quantify the changes in the histological structure [5, 6].

The thyroid glands were fixed in Bouin's solution, embedded in paraplast and cut into three parallel step serial sections, 6  $\mu\text{m}$  thick. One series was stained with PAS and light green, the other with hematoxylin-eosin and the third with the silver impregnation method according to Fernandez-Pasquale to present parafollicular cells. The pituitaries, fixed and embedded in the same way as the thyroids, were cut into two parallel step serial sections, 4  $\mu\text{m}$  thick. One was stained according to Cleveland-Wolf and the other with immunoperoxidase method using rabbit antiserum against human TSH to present the thyrotropic cells.

The stereological analysis of the two types of glands was carried out by a multipurpose test system (M-42) inserted into the eyepiece at a magnification  $\times 10$ . First, the total (absolute) volumes of the thyroid glands and distal parts of the adenohypophysis were determined at an objective magnification of  $\times 10$ . At an objective magnification  $\times 60$  the volume densities of the epithelial, parafollicular and thyrotropic cells were estimated. In addition, the numerical densities of their nuclei were estimated at an immersion objective magnification  $\times 100$ , using our procedure [7]. From the stereological values obtained the total as well as the average volumes of the cells and the total numbers of the cell nuclei were calculated. Finally, Student's *t* test was used for the statistical analysis.

US EPA, 1987

$$BW = 0.0353$$

$$C = 0.09 [BW^{0.75}] = 0.0063 \text{ L/day}$$

$$\text{Est. Dose} = \frac{1.2 \text{ g/kg}}{100 \text{ L}} \times \frac{0.0063 \text{ L}}{\text{day}} \times \frac{1}{0.0353 \text{ kg}} \times \frac{10^3 \text{ mg}}{1 \text{ g}} = 2147.1 \text{ mg/kg/day} = 2147 \text{ mg/kg/day} \quad 178$$

US EPA, 1987

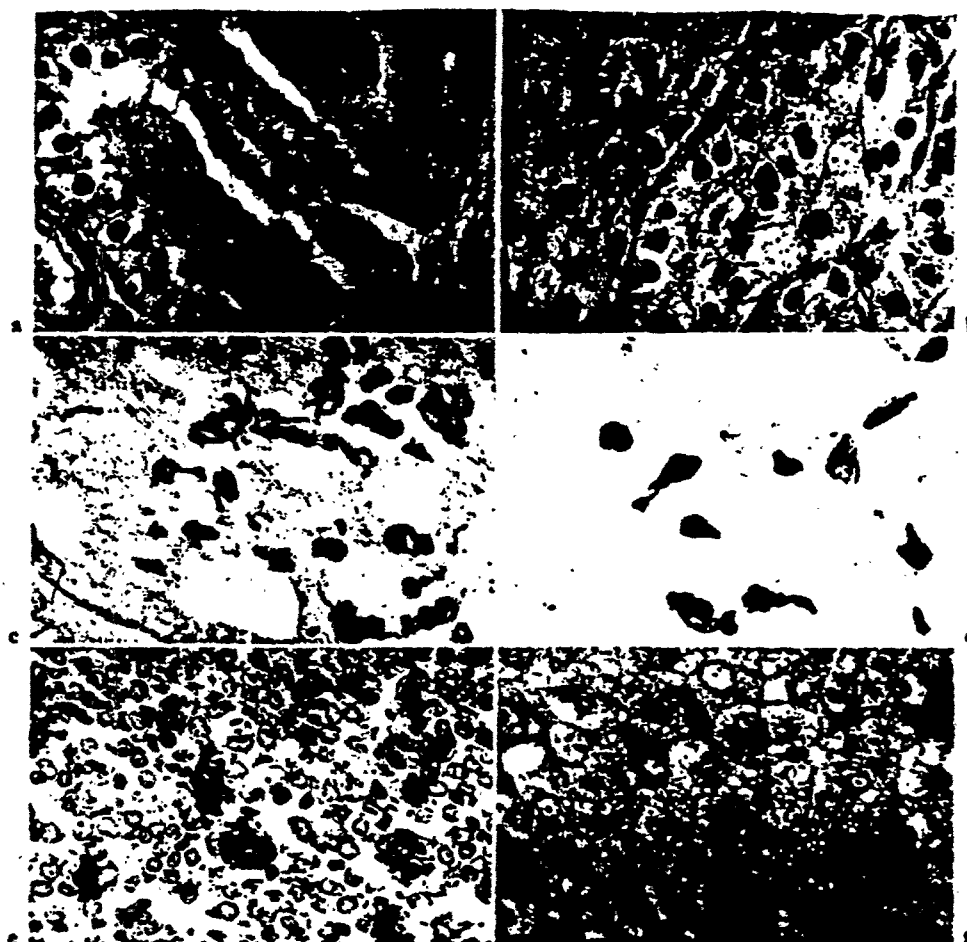


Fig. 1. Mouse thyroid glands of the control (a) and perchlorate (b) group stained by hematoxylin-eosin, thyroid parafollicular cells of the control (c) and perchlorate (d) group stained according to Fernandez-Pasquale, and pituitary thyrotropic cells of the control (e) and perchlorate (f) group stained by immunoperoxidase method with antihuman TSH serum.  $\times 40$ .

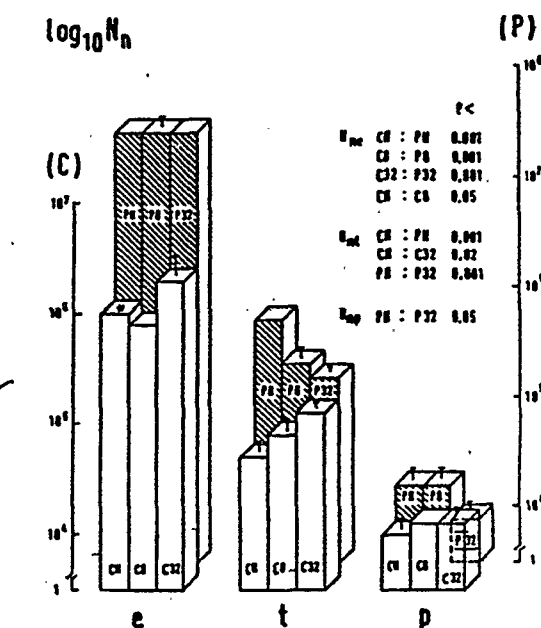
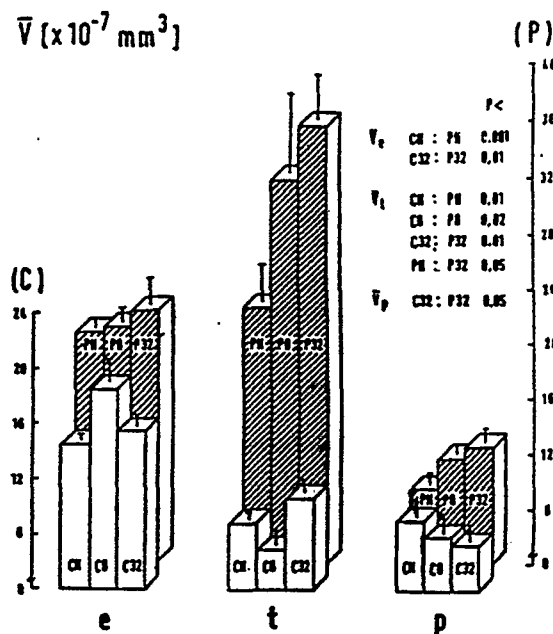
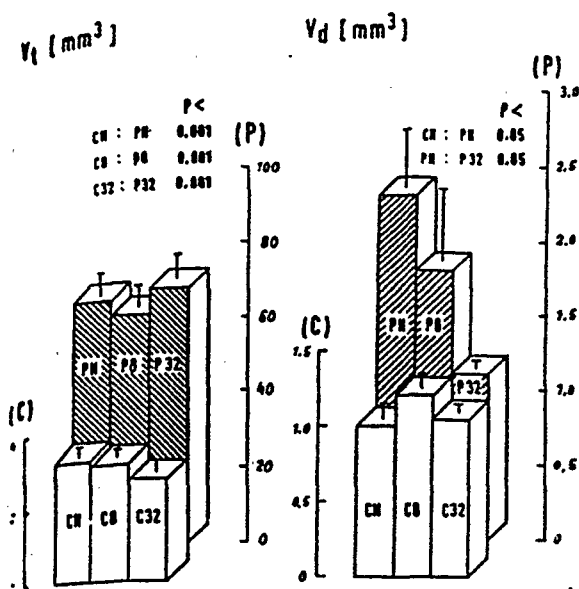
## Results

Obvious changes have to be seen in the histological pictures of the thyroid and pituitary glands after perchlorate drinking (fig. 1). The incidence of follicular cell carcinoma among 42 sacrificed animals was 0/22 in all control groups, 5/6 in perchlorate-non-irradiated and 14/14 in perchlorate-irradiated groups; no medullary carcinoma appeared. Stereological analysis after perchlorate application showed an average increase in the total volume of the thyroid glands to  $65 \text{ mm}^3$  in comparison to  $3 \text{ mm}^3$  in the controls. The total volume increase of the pituitary distal parts after perchlorate treatment was  $2.3 \text{ mm}^3$  in nonirradiated animals in comparison to  $1.1 \text{ mm}^3$  in nonirradiated controls. In late irradiated perchlorate animals this increase was negligible (fig. 2). Figure 3 presents the effect of perchlorate treatment in the

sense of cell hypertrophy documented in the average cell volumes of all three cell lines. The total number of cell nuclei, being an indicator of cell hyperplasia, was increased 20 times on average after perchlorate treatment in all epithelial cells. This increase after perchlorate was less expressed in the parafollicular and thyrotropic cells of nonirradiated animals. The irradiation annihilated this effect in the thyrotropic and parafollicular cells, especially when irradiation was used later (fig. 4).

## Discussion

In this study for the first time the effects of perchlorate application; ionizing irradiation and their interaction on the thyroid and pituitary glands have been quantitatively evaluated, systematically using



micrological methods. The impairment of the iodine pump by perchlorate in the long-term experiment on mice reduced the synthesis of the two thyroid hormones and caused hyperthyrotropinemia [unpubl. data]. A strong hypertrophy and enormous hyperplasia of the thyroid follicular and pituitary thyrotropic cells was accompanied by a high incidence of follicular cell carcinoma.

From our previous research based upon 1 month of perchlorate drinking, an approximately 3-fold increase in the total number of parafollicular cells was known [8]. Almost 1 year after the present experiment there was no hyperplasia of the parafollicular cells and no medullary cancer appeared. After total fractional irradiation with low doses of gamma rays no uniform changes in the structure and function of the two glands could be detected.

The combined effect of perchlorate and irradiation

caused follicular cell carcinoma in all cases; besides, decreased hyperplasia and emphasized hypertrophy of the parafollicular as well as the thyrotropic cells was shown, especially after late irradiation. It was assumed that the reduced total number of thyrotropic and parafollicular cell nuclei was the result of irradiation damage, partly compensated by the increased average cell volume. The differences between the early and late irradiated animals could be ascribed to the longer restoration time for the former animals, indicating the reversibility of the radiation damage.

### Acknowledgment

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Dr. Zdenka Pajer  
Institute of Histology and Embryology  
Medical Faculty  
Korytkova 2, PO Box 10  
YU-61105 Ljubljana (Yugoslavia)